IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Man *et al.*

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For: PHARMACEUTICALLY ACTIVE ISOINDOLINE DERIVATIVES

Attorney Docket No.: 9516-205-999
(CAM 501872-999204)

DECLARATION OF DR. HON-WAH MAN

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, HON-WAH MAN, PH.D., declare as follows:

1. I received my B.S. in chemistry National Taiwan University, Taiwan, in 1984. I received a Ph.D. in organic chemistry from Iowa State University, Ames, IA, in 1990.

2. From 1990 to 1994, I was a postdoctoral research associate and then a staff scientist at Washington State University. From 1994 to 1996, I was a postdoctoral fellow at DuPont Merck Pharmaceutical Company.

3. Since 1996, I have been employed by Celgene Corporation, the assignee of the above-identified patent application. During my tenure at Celgene Corporation, I held positions of a Research Scientist, a Senior Research Scientist, a Staff Scientist, and a Group Leader. Currently, I hold a position of an Associate Director at Celgene Corporation. I am named as a co-inventor of the above-identified patent application.

4. I have extensively published in peer-reviewed journals and made presentations at various academic conferences. My *curriculum vitae* is attached hereto as **Exhibit A**.

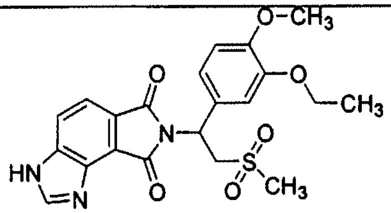
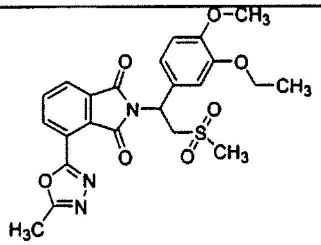
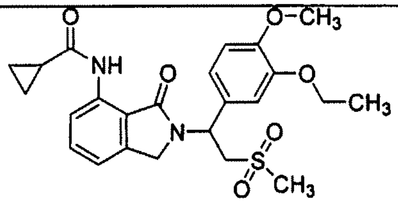
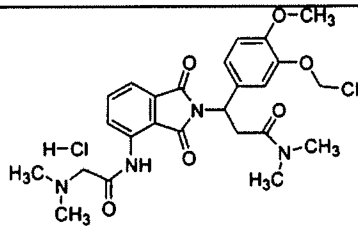
5. By training and experience, I am familiar with the evaluation of compounds having biochemical and pharmacological properties. In particular, in my capacity as an Associate Director at Celgene Corporation, I am familiar with the evaluation of compounds provided in the current application.

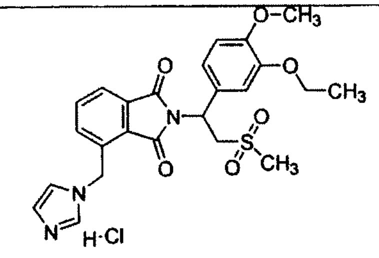
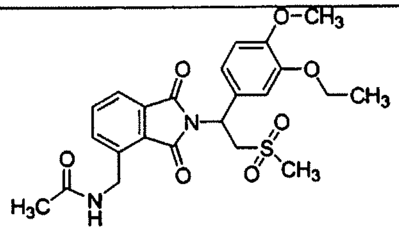
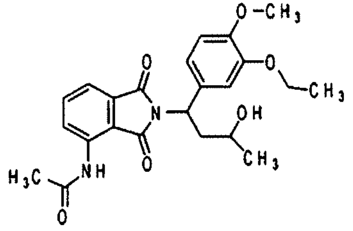
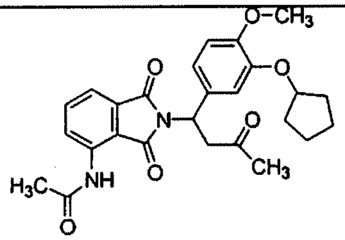
6. Tests were conducted at Celgene Corporation to evaluate the relative activities of the compounds provided in the current application in inhibiting the levels of phosphodiesterase 4 ("PDE4"). The procedures were performed as follows:

> Using a modification of the method of Hill and Mitchell [Hill and Mitchell, *Faseb J.*, 8, A217 (1994)], U937 cells (a human promonocytic cell line) were grown to 1×10^6 cells/mL and collected by centrifugation. A cell pellet of 1×10^9 cells was washed in phosphate buffered saline and then frozen at -70°C for later purification or immediately lysed in cold homogenization buffer (20mM Tris-HCl, pH 7.1, 3 mM 2-mercaptoethanol, 1 mM magnesium chloride, 0.1 mM ethylene glycol-*bis*-(β-aminoethyl ether)-N,N,N^1,N^1-tetraacetic acid (EGTA), 1 μM phenylmethylsulfonyl fluoride (PMSF), and 1 μg/mL leupeptin). Cells were homogenized with 20 strokes in a Dounce homogenizer and the supernatant containing the cytosolic fraction are obtained by centrifugation. The supernatant was then loaded onto a Sephacryl S-200 column equilibrated in homogenization buffer. The crude phosphodiesterase type 4 enzyme was eluted in homogenization buffer at a rate of approximately 0.5 mL/min and fractions were assayed for phosphodiesterase activity using rolipram. Fractions containing PDE 4 activity (rolipram sensitive) are pooled and aliquoted for later use.
>
> The phosphodiesterase assay was carried out based on the procedure described by Hill and Mitchell. The assay was carried out in a total volume of 100 μl containing various concentration of test compounds, 50mM Tris-HCl, pH 7.5, 5 mM magnesium chloride and 1 μM cAMP of which 1% is ^{3}H cAMP. Reactions were incubated at 30°C for 30 minutes and then terminated by boiling for 2 minutes. The amount of PDE 4 containing extract used for these experiments was predetermined such that reactions are within the linear range and consume less than 15% of the total substrate. Following termination of reaction, samples were chilled at 4°C and then treated with 10μL of 10 mg/mL snake venom for

15 minutes at 30°C. Unused substrate then was removed by adding 200μl of a quaternary ammonium ion exchange resin (AG1-X8, BioRad) for 15 minutes. Samples then were spun at 3000 rpm, 5 minutes and 50 μl of the aqueous phase were taken for counting. Each data point was carried out in duplicate and activity was expressed as percentage of control. The IC_{50}s of the compounds are then determined from dose response curves of a minimum of three independent experiments.

7. Using procedures described in paragraph 6 above, the IC_{50}s (in μM) of the compounds for PDE4 were determined and shown in the following table:

Example #	Structure	PDE 4 IC_{50} (μM)
3		0.356
15		0.658
55		0.278
61		0.287

65		0.095
66		0.105
71		0.543
76		0.198

8. As can be seen above, the compounds tested possess good IC_{50} values for PDE4. The results show that the compounds recited by the currently pending claims are indeed effective in inhibiting PDE4.

I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that I make those statements with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: July 30, 2010

HON-WAH MAN, PH.D.

EXHIBIT A

Hon-Wah Man

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Experience and Accomplishments

4/07-present **Associate Director** **Celgene Corporation**
4/04-4/07 **Group Leader**
4/02- 4/04 **Staff Scientist**
9/98-4/02 **Senior Research Scientist**
9/96-9/98 **Research Scientist**

- Main inventor of four compounds which were selected as development candidates. One compound is in Phase II clinical trials for the treatment of psoriasis, one compound is in Phase I, and two compounds are in pre-clinical stage.
- Conducted drug discovery research in inflammatory cytokines, Phosphodiesterase 4 (PDE4), and cancer.
- Designed and discovered PDE4 and TNF-α inhibitors, some of which are 10,000 fold more potent than the clinical candidate (CDC-801).
- Co-inventor of a series of orally active cytokine inhibitors.
- Main inventor of a series of orally active anti-tubulin inhibitors, which induce apoptosis in various human cancer cells.
- Supervised 2 MS and 2 PhD chemists.

9/94-9/96 **Postdoctoral Fellow** **DuPont Merck Pharmaceutical Company**
Advisor: Dr. Prabhakar K. (PK) Jadhav

- Conducted drug discovery research in HIV-1 protease inhibitor and process research in boronic acid type thrombin inhibitor.
- Completed two multi-step asymmetric syntheses of two novel molecules, cyclic oxamide and bicyclic urea, as HIV-1 protease inhibitors for AIDS treatment.
- Designed and developed a new catalytic enantioselective synthesis of α-chloroalkyl boronates with excellent asymmetric induction.
- Developed a new process for the starting material of DuP714, which potentially reduces 30% of the cost of manufacture.
- Discovered a direct and safe process for synthesis of air and moisture stable aminoboronate derivatives.

1/94-9/94 **Staff Scientist** **Washington State University**
10/90-1/94 **Postdoctoral Research Associate**
Professor: Dr. Donald S. Matteson

- Completed and patented a multi-step asymmetric synthesis of an insect attractant, Stegobinone. Resulted in a 3-year grant from the Washington Technology Center.
- Completed within 9 months a contract project sponsored by Cell Therapeutics, Inc. Designed and accomplished a multi-step synthesis of their chiral drug (ProTec^{TM}) using carbohydrate chemistry. Obtained a chemically and optically pure final product without chromatographic separation in the reaction sequence.
- Initiated several exploratory research projects: enolate addition of haloalkylboronate; asymmetric intramolecular cyclopropane and cyclobutane formation; reduction of haloalkylboronate; and retroracemization. Resulted in a 3-year grant from the NSF.
- Supervised 3 graduate and 1 undergraduate students.

4/89-10/90 **Research Assistant** **Northeastern University**
8/84-3/89 **Research Assistant and Teaching Assistant** **Iowa State University**
Professor: Dr. Philip M. Warner

- Studied novel polylithium compounds, prepared C13 isotope labeled compounds.

Education

1990	**Iowa State University**, Ames, Iowa, USA	Ph.D. Chemistry (Organic)	GPA 3.9/4.0
1984	**National Taiwan University**, Taiwan, ROC	B.S. Chemistry	Grade A

Publications and Patents

1. Discovery of (S)-N-{2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methanesulfonylethyl]-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl}acetamide (Apremilast), a Potent and Orally Active Phosphodiesterase 4 and Tumor Necrosis Factor-.alpha. Inhibitor: **Man, H.W.**; Schafer, P.; Wong, L.M.; Patterson, R.T.; Corral, L.G.; Raymon, H.; Blease, K.; Leisten, J.; Shirley, M.A.; Tang, Y.; Babusis, D.M.; Chen, R.S.; Stirling, D.; Muller, G.W. *J. Med. Chem.* **2009,** 52(6), 1522-1524.
2. The anti-cancer drug lenalidomide inhibits angiogenesis and metastasis via multiple inhibitory effects on endothelial cell function in normoxic and hypoxic conditions: Lu, L.; Payvandi, F.; Wu, L.; Zhang, L-H; Hariri, R. J.; **Man, H.W.**; Chen, R.S.; Muller, G.W.; Hughes, C.C.W.; Stirling, D.I.; Schafer, P.; Bartlett, J.B. Microvascular Res. **2009**, 7(2), 78-86.
3. Antiproliferative Effects of CC-8062 and CC-8075 in Pancreatic Cancer Cells: Mouratidis, P.X.E.; Colston, K.W.; Bartlett, J.B.; Muller, G.W.; Man, H-W; Stirling, D.; Dalgleish, A.G. Pancreas (Hagerstown, MD, United States), **2009**, 38(1), 78-84.
4. N-alkyl-hydroxamic acid-isoindolyl compounds and their pharmaceutical uses: **Man, H.W.**; Muller, G.W. **2005**, US Patent US 6911464.
5. Pharmaceutically active isoindoline derivatives: Muller; G.W.; **Man; H.W.**; Stirling; D.I. **2004**, US Patent US 6762195.
6. Acylhydroxamic acids and method of reducing inflammatory cytokine levels: **Man, H.W.**; Muller, G.W.; Huang, S.Y. **2004,** US Patent US 6699899.
7. Pharmaceutically active isoindoline derivatives: **Man, H.W.**; Muller, G.W. **2003**, US Patent US 6667316.
8. Method of inhibiting phosphodiesterases with substituted alkanohydroxamic acids: Muller, G.W.; **Man, H.W. 2003** US Patent US 6656964
9. Effect of 3-fluorothalidomide and 3-methylthalidomide enantiomers on tumor necrosis factor production and antitumor responses to the antivascular agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA). Chung, F.; Palmer, B.D.; Muller, G.W.; **Man, H.W.**; Kestell, P.; Baguley, B.C.; Ching, L.M. *Oncology Res.* **2003**, 14, *2*, 75.
10. Selective Cytokine Inhibitory Drugs with Enhanced Antiangiogenic Activity Control Tumor Growth through Vascular Inhibition: Gee, M.S.; Makonnen, S.; al-Kofahi, K.; Roysam, B.; Payvandi, F.; **Man, H.W.**; Muller, G.W.; Lee, W.M.F. *Cancer Res.* **2003**, 63, *23*, 8073.
11. α-Fluoro-substituted thalidomide analogues: **Man, H.W.**; Corral, L.G.; Stirling, D.I.; Muller, G.W. *Bioorg. Med. Chem. Lett.* **2003**, 13, 3415.
12. Enhancement of cytokine production and AP-1 transcriptional activity in T cells by thalidomide-related immunomodulatory drugs: Schafer, P.H.; Gandhi, A.K.; Loveland, M.A.; Chen, R.S.; **Man, H.W.**; Schnetkamp, P.P.M.; Wolbring, G.; Govinda, S.; Corral, L.G.; Payvandi, F.; Muller, G.W.; Stirling, D.I. *J. Pharm. Exp. Ther.* **2003**, 305, 1222.
13. A Novel Subclass of Thalidomide Analogue with Anti-Solid Tumor Activity in Which Caspase-dependent Apoptosis is Associated with Altered Expression of bcl-2 Family Proteins: Marriott, J.B.; Clarke, I.A.; Czajka, A.; Dredge, K.; Childs, K.; **Man, H.W.**; Schafer, P.; Govinda, S.; Muller, G.W.; Stirling, D.I.; Dalgleish, A.G. *Cancer Res.* **2003**, 63, *3*, 593.
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15. Substituted 1,3,4-Oxadiazoles and a method of reducing TNF-α levels: **Man, H.W.**; Muller, G.W. **2001,** US Patent US 6326388.
16. Substituted alkanohydroxamic acids and method of reducing TNF–α levels: Muller, G.W.; **Man, H.W. 2001**, US Patent US 6214857.
17. Potent inhibition of cytokine production from intestinal lamina propria T cells by phosphodiesterase-4 inhibitory thalidomide analogues: Prehn, J.L.; Landers, C.; Muller, G.W.; **Man, H.W.**; Stirling, D.I.; Targan, S.R. *J Clin. Immunol.* **2001**, 21 (*5*), 357.
18. Substituted phenethylsulfones and method of reducing TNF-α levels: Muller, G.W.; **Man H.W. 2000,** US Patent US 6020358.
19. Substituted phenethylsulfones and method of reducing TNF-α levels: Muller, G.W.; **Man H.W. 2000,** US Patent US 6011050.
20. Substituted 2-(2,6-dioxo-3-fluoropiperidin-3-yl)-isoindolines and method of reducing inflammatory cytokine levels: Muller, G.W.; Stirling, D.I.; Chen, R.S.C.; **Man, H.W. 1999**, US patent, US 5955476.
21. Substituted 2-(2,6 dioxo-3-fluoropiperidin-3-yl)-isoindolines and method of reducing TNF.alpha. levels: Muller, G.W.; Stirling, D.I.; Chen, R.S.C.; **Man, H.W. 1999**, US patent, US 5,874,448
22. A highly enantioselective and diastereoselective synthesis of cyclobutanes via boronic esters: **Man, H.W.**; Hiscox, W.C.; Matteson, D.S. *Org. Lett.* **1999,** *1*, 379.

23. Nonpeptide cyclic cyanoguanidines as HIV-1 protease inhibitors: synthesis, structure-activity relationship, and X-ray crystal structure studies: Jadhav, P.K.; Woerner, F. J.; Lam, P.Y.S.; Hodge, C.N.; Eyermann, C.J.; **Man, H.W.**; Daneker, W.F.; Bacheler, L.T.; Rayner, M.M.; Meek, J.L.; Erickson-Viitanen, S.; Jackson, D.A.; Calabrese, J.C.; Schadt, M.; Chang, C-H. *J. Med. Chem.* **1998,** *41,* 1446.
24. Substituted 2-(2,6-dioxo-3-fluoropiperidin-3-yl)-isoindolines and method of reducing TNF−α levels: Muller, G.W.; Stirling, D.I.; Chen, R.S.C.; **Man, H.W. 1997**, US Patent US 5874448.
25. Method for the stereocontrolled synthesis of stegobinone via borane intermediates: Matteson, D.S.; **Man, H.W.**; Ho, O.C. **1997**, US Patent US 5681978 A.
26. Synthesis of 7-membered cyclic hydroxyguanidines: novel HIV-1 protease inhibitors: Jadhav, P.K.; Woerner, F.J.; **Man, H.W.** *Bioorg. Med. Chem. Lett.* **1997**, 7, 2145.
27. Enantiotopic differentiation of pro-R or pro-S chlorides in (dichloromethyl)borates by chiral Lewis acids: enantioselective synthesis of (α−chloroalkyl)boronates: Jadhav, P.K.; **Man, H.W.** *J. Am. Chem. Soc.* **1997**, 119, 846.
28. Asymmetric Synthesis of Stegobinone via Boronic Ester Chemistry: Matteson, D.S.; **Man, H.W.**; Ho, O.C. *J. Am. Chem. Soc.* **1996**, 118, 4560.
29. Synthesis of 7-membered cyclic oxamides: novel HIV-1 protease inhibitors: Jadhav, P.K.; **Man, H.W.** *Tetrahedron Lett.* **1996**, 37, 1153.
30. Direct synthesis of 1-*t*-butoxycarbonylaminoalkylboronates from (α−haloalkyl)boronates: Jadhav, P.K.; **Man, H.W.** *J. Org. Chem.* **1996**, 61, 7951.
31. Hydrolysis of substituted 1,3,2-dioxaborolanes and an asymmetric synthesis of a differentially protected syn,syn-3-methyl-2,4-hexanediol: Matteson, D.S.; **Man, H.W.** *J. Org. Chem.* **1996**, 61, 6047.
32. Enantioselective capture and retroracemization of (1-bromoalkyl)boronic esters by an N-propanoyloxazolidinone enolate and iodide ion: Matteson, D.S.; **Man, H.W.** *J. Org. Chem.* **1994**, 59, 5734.
33. High precision asymmetric synthesis of stegobiol and stegobinone via boronic esters: Matteson, D.S.; **Man, H.W.** *J. Org. Chem.* **1993**, 45, 6545.

Awards

- Thomas Alva Edison patent awards, Research and Development Council of New Jersey, 2007
- Celgene Revlimid contributor award 2005
- Industrial Scholarship, Iowa State University, 2 yrs.
- Book Coupon Award (top five percent of the class), National Taiwan University, 3 semesters.
- Excellence Overseas Student Award, Taiwan, 2 yrs.